Prep1 (Pknox1) is a homeodomain transcription factor in theTALE superfamily and a critical regulator of hematopoiesis and organogenesis. Although *Prep1* null mice die at gastrulation, we found that a genetic combination of a *Prep1* null allele with a hypomorphic allele ($Prep1^{i/-}$) rescues embryonic viability until mid-gestation allowing new insights into its function in early development. Severe $Prep1^{i/-}$ embryos show complex and dramatic anterior phenotypes, including anophthalmia or microphthalmia. The primary ocular phenotype appears to be complete absence of the lens, and analyses of lens development revealed that lens induction failed in $Prep1^{i/-}$ embryos, accompanied by decreased or absent Pax6 and Foxe3 expression. Prep1 was required for *Pax6* ectodermal enhancer (EE) activity, but not pancreatic enhancer activity. Using protein-binding microarray analysis, we identified two tandem highly conserved Prep1 bindings sites in the *Pax6* EE, predicted to be of medium-affinity binding strength. These affinity differences were verified using biophysical real-time measurements of protein/DNA interactions. Mutation of either site abrogated *Pax6* EE reporter activity, which could be rescued by mutagenesis of one of the sites to a high-affinity Prep1 binding site. Therefore, Prep1 is a novel regulator of lens induction and a newly identified and critical upstream regulator of Pax6. Moreover, we define a novel cis-regulatory logic, whereby medium-affinity transcription factor binding sites act additively to define a genetic threshold for gene activation.

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Program/Abstract # 178
Roles of zebrafish Nipped-B-like (Nipbl) in gene regulation
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Heterozygous loss of *Nipped-B-like* (*NIPBL*) is the most common cause of Cornelia de Lange Syndrome (CdLS). Nipbl has a highly conserved role in loading cohesin onto chromosomes, but studies in *Drosophila* suggest it also exerts genome-wide effects on gene expression. *Nipbl*+/− mice display CdLS-like phenotypes, without chromosome cohesion defects (unpublished data), suggesting that CdLS results from dysregulated gene regulation. To investigate how Nipbl affects gene expression, we disrupted Nipbl expression in zebrafish embryos. Zebrafish has two genes, *zNipbl-1* and *zNipbl-2*, with >70% amino acid identity to mammalian Nipbl; both are expressed maternally and ubiquitously in early embryos. Knock-down of either *zNipbl* with antisense morpholinos (MOs) led to gross morphological defects by 12 hpf; effects were more severe when both genes were knocked down. To identify the earliest transcriptional changes due to loss of Nipbl function, microarray analyses were performed on morphants at gastrula stages (6.5–9.0 hpf). When both zNipbls were depleted with translation-blocking MOs, at least 7 genes were significantly up-regulated by 6.5 hpf, just hours after the mid-blastula transition (MBT, onset of zygotic transcription). Maternal mRNAs for all 7 are found in embryos and normally degraded after MBT. Preliminary data suggest that it is not stabilization of maternal mRNAs, but inappropriate onset of zygotic transcription, that accounts for their up-regulation in zNipbl morphants. Experiments are underway to elucidate the effects of Nipbl on the transcription of these genes. Supported by the NIH P01-HD052860.

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Program/Abstract # 179
Imprinting analysis in the Acrodysplasia region of mouse chromosome 12
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The insertional mouse mutation *Acrodysplasia* (*Adp*) confers a parent-of-origin developmental phenotype, with animals inheriting the mutation from their father showing skeletal abnormalities, while those inheriting the mutation from their mother are normal. This parental-specific phenotype, along with mapping of the insertion to a region of chromosome 12 proposed to contain imprinted genes, suggested that disruption of genomic imprinting might underlie the *Adp* phenotype. Genomic imprinting is the process by which autosomal genes are epigenetically silenced on one of the two parental alleles; imprinting mutation phenotypes manifest after inheritance from one parent but not the other. Imprinted genes typically occur in dense clusters that contain few nonimprinted genes, and therefore assaying representative genes from the *Adp* critical region might identify any imprinted domains. Thirteen genes spaced across the *Adp* region were analyzed for imprinting, but all were found to be biallelically expressed. Other explanations must therefore be considered for the parent-of-origin *Adp* phenotype.

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Program/Abstract # 180
Molecular mechanism of wound-dependent Grh regulation
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Abstract #180 will be presented as scheduled, but will not be published due to lack of license agreement between authors and publisher.

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Program/Abstract # 181
Serine protease activation of epidermal wound response genes in *Drosophila*
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Following epidermal damage, breaches in the skin or cuticle are repaired by the epidermal wound response, a survival mechanism that restores barrier integrity. However, the manner in which nearby unwounded epidermal cells sense the wound and begin the process of repair is unknown. Here we show that serine proteases and their inhibitors are involved in regulating the expression of wound response genes encoding Dopa decarboxylase (Ddc), a cuticular crosslinking enzyme and misshapen (msn), a kinase that activates JNK signaling that is crucial for epidermal tissue movement and fusion. Transgenic flies containing reporter constructs fused to upstream enhancer sequences of Ddc and msn were generated to monitor transcriptional expression after wounding. After simultaneous aseptic wounding and injection of the broad-spectrum serine protease trypsin into late stage embryos, widespread epidermal Ddc expression was observed. Similar wounding and injection of the serine protease inhibitor aprotinin resulted in highly reduced expression levels of msn surrounding the wound site. Aseptic wounding and injection of papain, a cysteine protease, seemed to have no effect on the normal wound response expression of msn, suggesting that the wound response is serine protease specific. Serine proteases have been found to play important wound healing roles in a variety of epithelial tissues. These enzymes also activate melanization events after septic wounding. Our results indicate that serine proteases can activate specific wound response genes following epidermal damage.

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